ISSN (print) 0093 4666 © 2011. Mycotaxon, Ltd. ISSN (online) 2154 8889

MYCOTAXON

Volume 115, pp. 507 520 *January March 2011*

DOI: 10.5248/115.507

Morphological studies in *Dothideomycetes: Elsinoe* (*Elsinoaceae*), *Butleria,* and three excluded genera

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Abstract The types of the genera *Beelia*, *Butleria*, *Elsinoe*, *Hyalotheles*, and *Saccardinula* were examined to revise their familial position. The family *Elsinoaceae* (type: *Elsinoe canavaliae*) is described and its separation from *Myriangiaceae* is supported. *Butleria inaghatahani* has characters similar to *Elsinoaceae* where it should remain. *Beelia suttoniae* appears to be a superficial biotroph on the surface of leaves and thus *Beelia* should be placed in *Chaetothyriaceae* and is most similar to *Ainsworthia* (*Phaeosaccardinula*). Apart from the oblong to ovoid sessile asci in *Hyalotheles dimerosperma*, its placement in *Elsinoaceae* seems unwarranted, and *Hyalotheles* should be placed in *Dothideomycetes* incertae sedis. *Saccardinula guaranitica* may be better placed in *Microthyriaceae* or *Brefeldiellaceae*, because its ascomata greatly resemble thyrothecia found in *Microthyriaceae* and have similarities with *Brefeldiella*. Molecular sequence data from fresh collections is required to solve the problem of familial placement.

Key words — *Ascomycota*, morphology, taxonomy

Introduction

We are conducting studies on the *Dothideomycetes* in order to provide a natural classification (Zhang et al. 2008, 2009; Wu et al. 2010). As part of this research, we are restudying the type species of genera placed in the *Elsinoaceae*, a poorly known but relatively important family within the *Dothideomycetes* (Lumbsch & Huhndorf 2007). The *Elsinoaceae* presently comprise 10 genera including *Beelia*, *Butleria*, *Elsinoe*, *Hemimyriangium*, *Hyalotheles*, *Micularia*, *Molleriella*, *Saccardinula*, *Stephanotheca,* and *Xenodium* (Lumbsch & Huhndorf 2007). The family is characterized by immersed or erumpent ascomata,

composed of pale gelatinous thin-walled hyphal or pseudoparenchymatous cells. Asci are arranged in individual locules, in a single layer or are irregularly scattered, and are saccate to globose, bitunicate and fissitunicate. Ascospores are hyaline to brown, septate, or sometimes muriform (Kirk et al. 2001, 2008). The known anamorphs in *Elsinoaceae* are acervular coelomycetes with polyphialidic conidiogenous cells (e.g. *Sphaceloma* anamorphs of *Elsinoe*) (Sivanesan 1984). We have thus far examined several taxa within *Elsinoaceae* and in this paper report on the type species of *Elsinoe* and four poorly known genera: *Elsinoe canavaliae* (Raciborski 1900), *Beelia suttoniae* (Stevens 1925), *Butleria inaghatahani* (Saccardo 1914), *Hyalotheles dimerosperma* (Spegazzini 1908), and *Saccardinula guaranitica* (Spegazzini 1885). Full descriptions of these taxa and suggestion for their taxonomic placement are provided.

Materials & methods

Type specimens of *Elsinoe canavaliae*, *Beelia suttoniae*, *Butleria inaghatahani*, *Hyalotheles dimerosperma*, *Saccardinula guaranitica* were obtained from ZT & K, BISH, PAD, LPS and LPS, respectively. Ascomata were rehydrated in 3% KOH prior to examination and sectioning. Specimens were examined under a stereo microscope (Leica MZ16A) and fine forceps were used to remove one or two ascomata, which were mounted in water, Melzer's, Congo red, or cotton blue reagents. Observations and photographs were made under the light microscopes (Nikon E800 and Leica DM3000). Differential interference contrast microscopy was used for some hyaline structures.

Hand sections were cut with a sharp razor blade and thin (8 µm) sections were cut using a Leica CM1100 freezing microtome. The sections were transferred to a drop of water or a drop of cotton blue for examination and photography.

Taxonomy

Elsinoaceae Höhn. ex Sacc. & Trotter, Syll. fung. 22: 584 (1913).

Parasitic on leaves causing scabs and anthracnose. Ascomata immersed to erumpent, round or elongate, usually crustose, composed of pale gelatinous thin-walled hyphal or pseudoparenchymatous cells, opening by unordered breakdown of the surface layers. Specialized interascal tissue absent. Asci arranged in individual locules, in a single layer or irregularly arranged, saccate to globose, bitunicate, fissitunicate. Ascospores hyaline to brown, septate, sometimes muriform. Anamorph acervular where known.

Elsinoaceae was validated by Saccardo & Trotter (1913) based on the invalid non-Latin name "Elsinoëen" published by Höhnel (1909: 373). Höhnel (1909) regarded *Elsinoaceae* as a separate family at the same level with *Myriangiaceae* among "die Protodiscineen" or "die Plectascineen". Von Arx & Müller (1975) extended the family concept and placed *Elsinoe* with another 15 genera in *Myriangiaceae*. Luttrell (1973) used this concept to unite seven additional genera with *Elsinoe* in *Myriangiaceae*. Barr (1979) and Eriksson (1981) maintained

the *Elsinoaceae* as separate from *Myriangiaceae,* mainly based on habit and developmental studies. Lumbsch & Huhndorf (2007) placed *Elsinoaceae* in the order *Myriangiales* and this is supported in the studies of Schoch et al. (2006, 2009) and Boehm et al. (2009). Further molecular study is needed to establish whether *Elsinoaceae* and *Myriangiaceae* are distinct families, although the evidence points towards this (Schoch et al. 2009).

TYPE GENUS: ***Elsinoe*** Racib., Paras. Alg. Pilz. Java's (Jakarta) 1: 14 (1900).

Ascomata parasitic, usually forming scabs or anthracnose on leaves. Ascomata pulvinate, white or occasionally brown, in section with numerous locules distributed inside the ascostromata, with numerous asci inside each locule. Paraphyses absent. Asci 8-spored, bitunicate, globose to subglobose, without a pedicel. Ascospores hyaline, transseptate or muriform.

ANAMORPH: *Sphaceloma* spp. Acervuli pseudoparenchymatous from which hyaline to pale-brown phialidic conidiophores and/or conidiogenous cells originate. Conidia hyaline, unicellular, ellipsoidal, biguttulate.

TYPE SPECIES: ***Elsinoe canavaliae*** Racib., Paras. Alg. Pilze Java's 1: 14 (1900), as "*cavavalliae*". FIG. 1

Ascomata parasitic on leaves, forming scabs on lower surface (FIG. 1A). Ascomata pulvinate, white or occasionally brown, 1.8–3.3 mm in diam., gregarious, immersed or erumpent, irregularly shaped, spreading around the host veins (FIG. 1B–C), in section with numerous locules distributed inside the ascostromata, with numerous asci inside each locule, with lower part of ascomata fusing with the hyaline host cells (FIG. 1D–E). Paraphyses not seen, probably absent. Asci 19–23 × 16–20 µm (mean = 21 × 18 µm, n = 10), 8-spored, bitunicate, globose to subglobose, without a pedicel, with a wide but indistinct ocular chamber (FIG. 1F–H). Ascospores 14–18 × 6–9 µm (mean = 15 × 7 µm, n = 10), irregularly arranged, hyaline, ellipsoidal-fusiform, 3–4 septate, constricted at the central septum, upper part wider with slightly acute ends, lower part narrow with rounded ends, guttulate (FIG. 1I–J).

ANAMORPH: *Sphaceloma.*

SPECIMEN EXAMINED: INDONESIA, JAVA, on leaves of *Canavalia gladiata* (Savi) DC. (*Fabaceae*) leg. Raciborski (ZT Myc 1489, lectotype; K 164015, syntype).

Elsinoe was established by Raciborski (1900) with descriptions of three species (*E. canavaliae, E. antidesmae* Racib., *E. menispermacearum* Racib.). Von Arx & Müller (1975) placed *Elsinoe* in *Myriangiaceae* based on its immersed or erumpent, pulvinate or irregular ascomata and being parasitic on the leaves of higher plants causing scabs. Later, the genus was removed to the family *Elsinoaceae* (Barr 1979, Lumbsch & Huhndorf 2007). The more than 141 species recorded for *Elsinoe* (www.indexfungorum.org 2010) are generally parasites on leaves, stems, scale insects or other fungi.

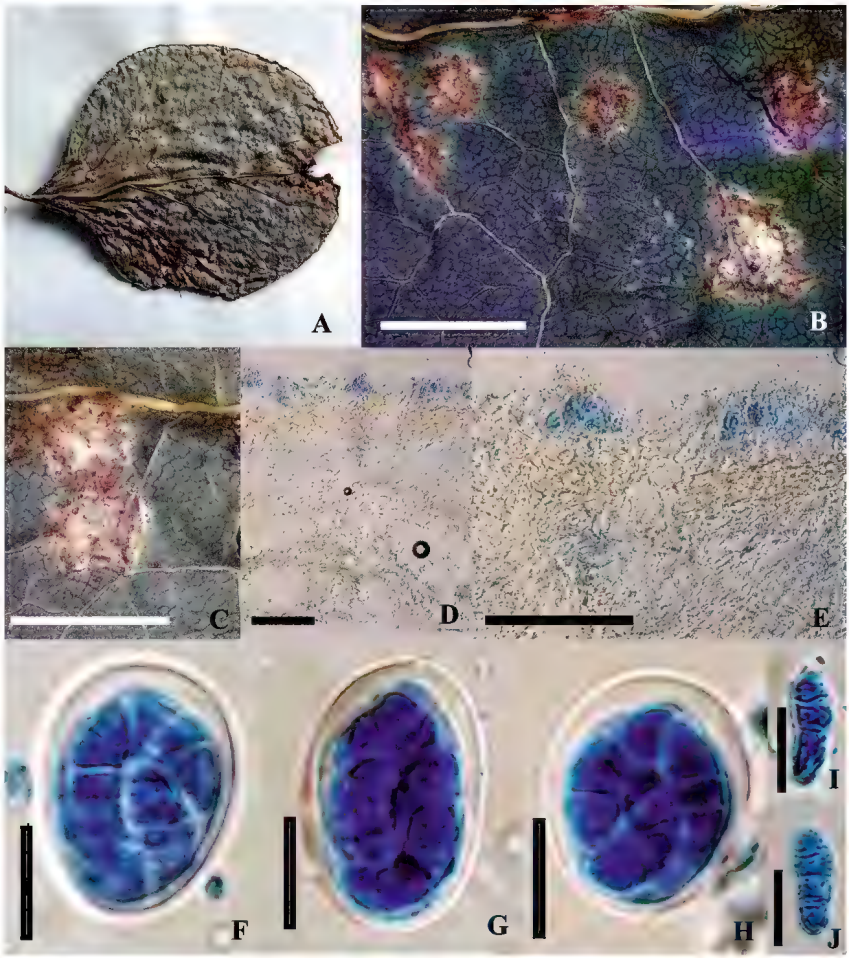


FIG. 1. *Elsinoe canavaliae* (lectotype) A. Appearance of ascomata on the host surface of leaves. B–C. Appearance of ascomata on the leaves showing the irregular shape of scabs. D–E. Vertical section through ascomata in cotton blue showing the ascomata with numerous asci inside each locule. F–H. Sessile asci in cotton blue. I–J. Ascospores in cotton blue.
Scale bars: B– C 5 mm, D– E 100 µm, F–J 10 µm.

This is an important plant pathogenic genus causing scab and anthracnose of *Citrus, Malus, Rubus, Vitis* spp. and other hosts and descriptions and disease symptoms can be found on the worldwide web. It is important however, to characterize the type of the genus, which is less well known. *Elsinoe fawcettii* Bitanc. & Jenkins and *E. australis* Bitanc. & Jenkins cause scab diseases of *Citrus* species (Hanlin 1989; Timmer et al. 1996), and there are many other important pathogens in this genus. Examples include *Elsinoe veneta* (Burkh.)

Jenkins, which causes cane spot of raspberry (Munro 1988); *E. dracophylli* P.R. Johnston & Beever, which causes spots on *Dracophyllum* (Johnson and Beever 1994); and *E. mangiferae* Bitanc. & Jenkins (Bitancourt & Jenkins 1946), which causes mango scab. *Elsinoe takoropuku* G.S. Ridl. & Ramsfield is a recently introduced species (Ridley & Ramsfield 2006), but it differs quite markedly from *E. canavaliae* as it does not cause scabs on leaves, but forms ascostromata on twigs with locules each containing single asci. The asci are thought to be more like the *Elsinoe* type, although the fungus shares many characters with *Myriangiaceae*, illustrating a clear need for molecular studies on this group.

There have been a few molecular studies incorporating strains of *Elsinoe*. Schoch et al. (2006, 2009) and Boehm et al. (2009) showed the species to cluster in the *Myriangiales* and form a distinct subclade – the *Elsinoaceae*. However, since only four *Elsinoe* specimens and one *Myriangium* specimen were used in Schoch et al. (2006) and no *Elsinoe* specimens were used in Schoch et al. (2009), the molecular data does not conclusively resolve two separate families (*Elsinoaceae* and *Myriangiaceae*). Swart et al. (2001) analyzed ITS sequence data of six *Elsinoe* species — *E. banksiae*, *E. leucospermi*, *E. proteae*, *Elsinoe* sp. (from *Citrus*), *Elsinoe* sp. (from *Banksia*), *Sphaceloma protearum* — in their research on the taxonomy of species associated with scab disease of *Proteaceae;* their molecular analyses supported five species, of which four were described in that paper. A molecular study of many more *Elsinoe* species is needed.

Beelia F. Stevens & R.W. Ryan, in Stevens, Bulletin of the Bernice P. Bishop Museum 19: 71 (1925).

Forming colonies on the surface of the leaves. Ascomata black, superficial, ostiolate, aparaphysate, borne on free, brown, septate, branching mycelium. Asci bitunicate, broadly ellipsoidal, obovate to saccate, pedicellate. Ascospores cylindrical, hyaline to straw-coloured, 6-celled.

Anamorphs unknown.

Type species: ***Beelia suttoniae*** F. Stevens & R.W. Ryan, in Stevens, Bulletin of the Bernice P. Bishop Museum 19: 71 (1925). Fig. 2

Ascomata on the upper surface of leaves, scattered beneath and between darkened mycelial strands (Fig. 2A–B). Ascomata 190–210 µm wide × 115–133 µm high, superficial, globose to subglobose, black, with a flattened base which is easily removed from the substrate containing numerous asci (Fig. 2C–D). Peridium 25–30 µm wide, up to 39 µm wide at the apex, 20 µm wide at the base; comprising two cell types; outer cells brown, thick-walled, textura globulosa, inner cells thin-walled, lighter, textura angularis (Fig. 2D–E). Paraphyses not seen, probably absent. Asci 70–89 × 45–55 µm (mean = 84.3 × 51.2 µm, n = 20), 8-spored, bitunicate, broadly ellipsoidal, obovate to saccate, thick-walled, with small pointed pedicle, and with ocular chamber up to 24.4 µm wide × 14.9 µm

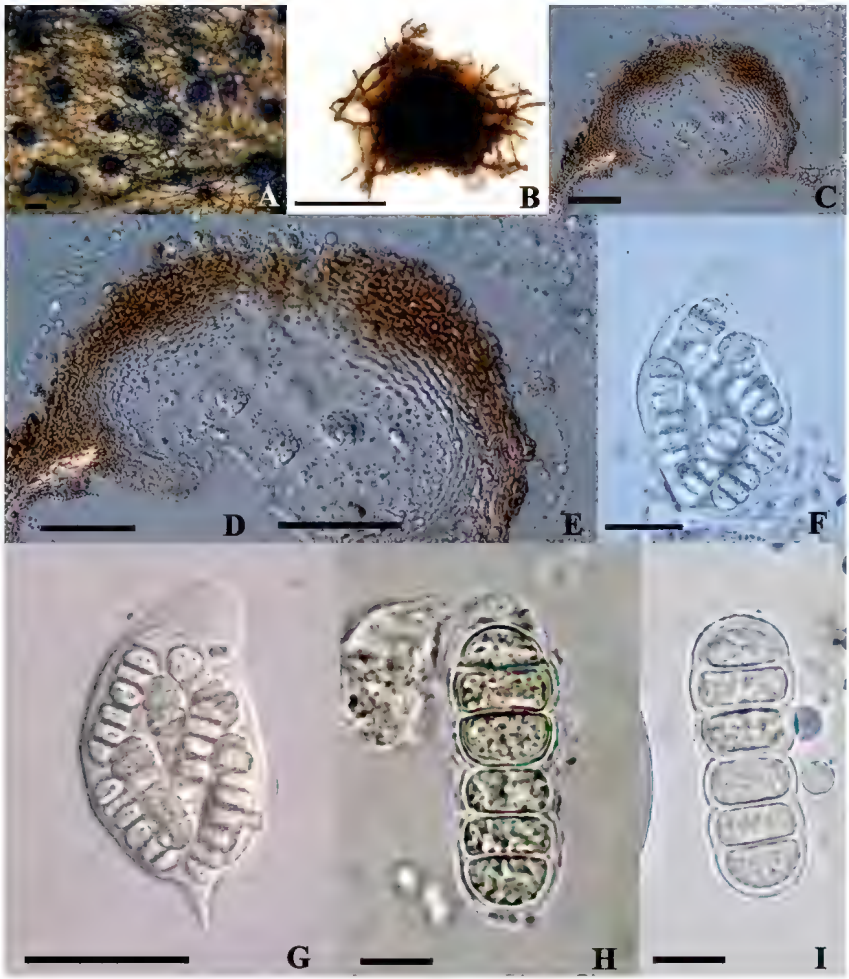


FIG. 2. *Beelia suttoniae* (syntype): A. Appearance of ascomata on host leaf scattered beneath and between darkened mycelium. B. Squash of ascoma in water. C–E. Vertical section through ascoma showing outer and inner cell types. F–G. Asci. Note the bitunicate appearance and ocular structure in the extended apex. H–I. Hyaline ascospores with 5 septa. Note the central septum is strongly constricted and upper part wider.

Scale bars: A, B 100 µm. C–E 50 µm, F 25 µm. G 50 µm. H–I 10 µm.

high (FIG. 2F–G). Ascospores 38–45 × 13–18 µm (mean = 42.8 × 14.6 µm, n = 20), irregularly arranged, cylindrical, hyaline, 5-septate, strongly constricted at each septum, central septum very strongly constricted and upper part wider, smooth-walled, with narrow mucilage sheath (FIG. 2 H–I).

ANAMORPH unknown.

Specimen examined: USA, Hawaii, on leaves of *Suttonia lanaiensis* Mez (*Myrsinaceae*), circa 1925, Lanai, no. 421, leg. Munro (BISH 499845, syntype).

Beelia was introduced by Stevens & Ryan (Stevens 1925) and remained monotypic until *B. philippinensis* Bat. & C.A.A (Batista & Costa 1959) and *B. plumeria* Bat. & Cavalc. (Batista et al. 1967) were added. Stevens (1925) placed *Beelia* in the family *Microthyriaceae,* where it was accepted by Petrak (1953). Von Arx & Müller (1975) transferred *Beelia* to the *Myriangiaceae* based on its dimidiate ascomata and long (> 30 µm) brown ascospores. Hawksworth et al. (1995) later transferred the genus to the *Elsinoaceae,* a placement accepted by Kirk et al. (2001) and Lumbsch & Huhndorf (2007). The taxon appears to be a superficial biotroph on leaf surfaces, a character shared by genera in *Chaetothyriaceae.* As *Beelia* seems most similar to *Ainsworthia* (Batista & Ciferri 1962; = *Phaeosaccardinula* Henn., fide von Arx & Müller 1975) in that family, we suggest that the genus may belong in *Chaetothyriaceae.* New collections and molecular analyses are needed to clarify the familial placement.

Butleria Sacc., Annls mycol. 12(3): 302 (1914).

Forming leaf spots. Ascomata gregarious, superficial, black, subglobose, with numerous locules distributed at different levels inside the ascomata, with only one globose to oblong asci inside each locule. Asci 8-spored, bitunicate, sessile. Ascospores oblong to ovoid, brown, two-celled.

Anamorphs unknown.

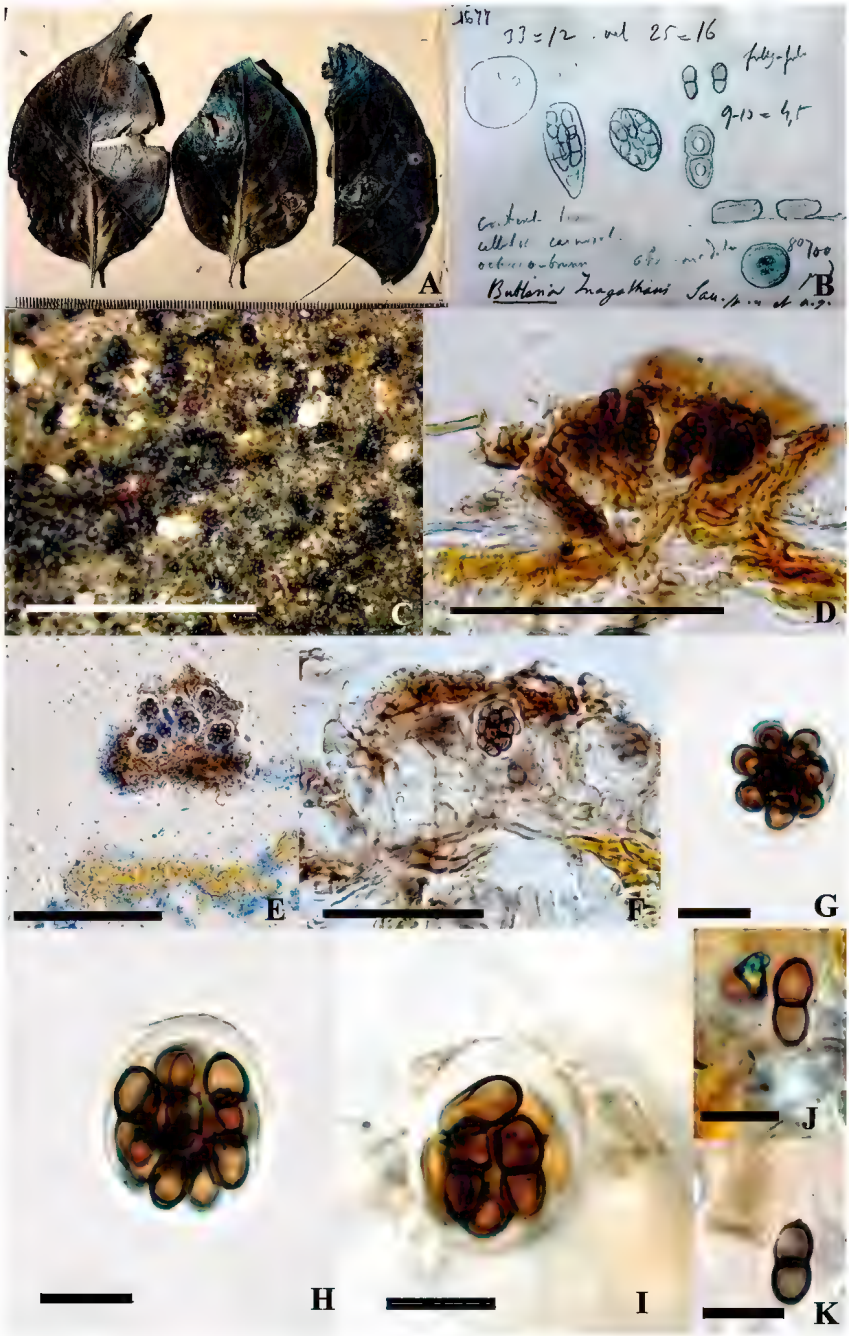
Type species: ***Butleria inaghatahani*** Sacc., Annls mycol. 12(3): 302 (1914). Fig. 3

Forming light, somewhat zonate target spots on leaves (Fig. 3A,C) with minutely stromatic ascomata forming on the upper surface. Ascomata 81–130 × 60–88 µm (mean = 114.4 × 75.2 µm, n = 15), gregarious, superficial, ovoid, subglobose to globose, black, with numerous locules distributed at different levels inside the ascomata, with only one globose to oblong asci inside each locule (Fig. 3B, D–F). Paraphyses not seen. Asci 19–25 × 16–23 µm (mean = 22.7 × 19.2 µm, n = 15), 8-spored, bitunicate, globose to oblong, sessile, with small ocular chamber up to 11.8 µm wide × 2.9 µm high (Fig. 3G–I). Ascospores 8–13 × 4–6 µm (mean = 12.1 × 4.8 µm, n = 15), irregularly arranged in rows of 3 or 4, oblong to ovoid, brown, 2-celled, constricted at the central septum, upper cell slightly larger than lower cell, spinulose (Fig. 3J–K).

Anamorph: unknown.

Specimen examined: BANGLADESH, Comillae District, Krishnapone, associated with target spots on leaves of *Vangueria* sp. (*Rubiaceae*), causing, 8 December 1913, leg. Inaghataban (PAD 1677, holotype).

Butleria is a monotypic genus established by Saccardo (1914) for *B. inaghatahani.* Von Arx & Müller (1975) referred this genus to *Myriangiaceae* based on its small bright ascomata and 2-celled ascospores. Currently, *Butleria* is placed in the family *Elsinoaceae* (Barr 1979; Lumbsch & Huhndorf 2007; Kirk et al. 2001,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2008). This taxon does appear to be a candidate for *Elsinoaceae* as it is a parasite on leaves and has single asci locules scattered throughout a somewhat reduced but pulvinate ascomata. The ascospores are, however, distinct in being brown. Here also additional collections and molecular analyses are needed to clarify family relationships.

Hyalotheles Speg., Revta Mus. La Plata 15(2): 11 (1908).

Forming black spots on the upper surface of leaves. Individual ascomata scattered, superficial, globose, black. Paraphyses not seen. Asci 8–spored, bitunicate, oblong to obovoid, without a pedicel. Ascospores irregularly arranged in 2---3 rows, globose, hyaline, 1-celled, minutely guttulate.

Anamorph: unknown.

Type species: ***Hyalotheles dimerosperma*** Speg., Revta Mus. La Plata 15(2): 11 (1908). Fig. 4

Forming black spots on the upper surface of leaves (Fig. 4A,C). Individual ascomata scattered, superficial, globose, black, composed of the hyaline pseudoparenchymatous cells at the edge and brown to dark pseudoparenchymatous cells at the center (Fig. 4B,D–E). Ascomata in section 50–62 × 54–60 µm (mean = 56 × 55 µm, n = 10) (Fig. 4E–F). Peridium dark to brown, up to 4–5 µm wide (Fig. 4E-F). Paraphyses not seen. Asci 23–28 × 15–18 µm (mean = 26 × 16 µm, n = 10), 8-spored, bitunicate, oblong to obovoid, without a pedicel (Fig. 4G–H). Ascospores 5–7 × 5–7 µm (mean = 5.5 × 6. µm, n = 8), irregularly arranged in 2–3 rows, globose, hyaline, 1-celled, minutely guttulate (Fig. 4I).

Anamorph: unknown.

Specimen examined: BRAZIL, Sao Paulo, Casa do Isolamento, on leaves of *Rubus urticifolius* Poir. (*Rosaceae*), collector unknown (LPS 408, holotype).

Hyalotheles is a monotypic genus introduced by Spegazzini (1908) for *H. dimerosperma*. The genus is characterized by ascomata developing on glandular hairs of the host and spherical ascospores (von Arx & Müller 1975). Von Arx & Müller (1975) placed *Hyalotheles* in the family *Myriangiaceae,* but later Barr (1979) transferred the genus to the family "*Saccardinulaceae*" [an invalid name]. Lumbsch & Huhndorf (2007) now recognize *Hyalotheles* in the family *Elsinoaceae*. Apart from the oblong to ovoid sessile asci, however, placement in *Elsinoaceae* seems unwarranted, and *Hyalotheles* is better referred to *Dothideomycetes* incertae sedis. Additional collections and molecular analyses are needed to clarify family relationships.

Fig. 3 (left). *Butleria inaghatahani* (holotype): A, C. Appearance of ascomata in leaf spot. B. Drawing from herbarium specimen. D, F. Vertical section through ascomata showing the part connected to the leaf. E. Vertical section through ascomata (in cotton blue reagent). G I. Saccate asci. J K. Ascospores. Scale bars: C 1 mm, D E 100 µm, F 50 µm, G K 10 µm.

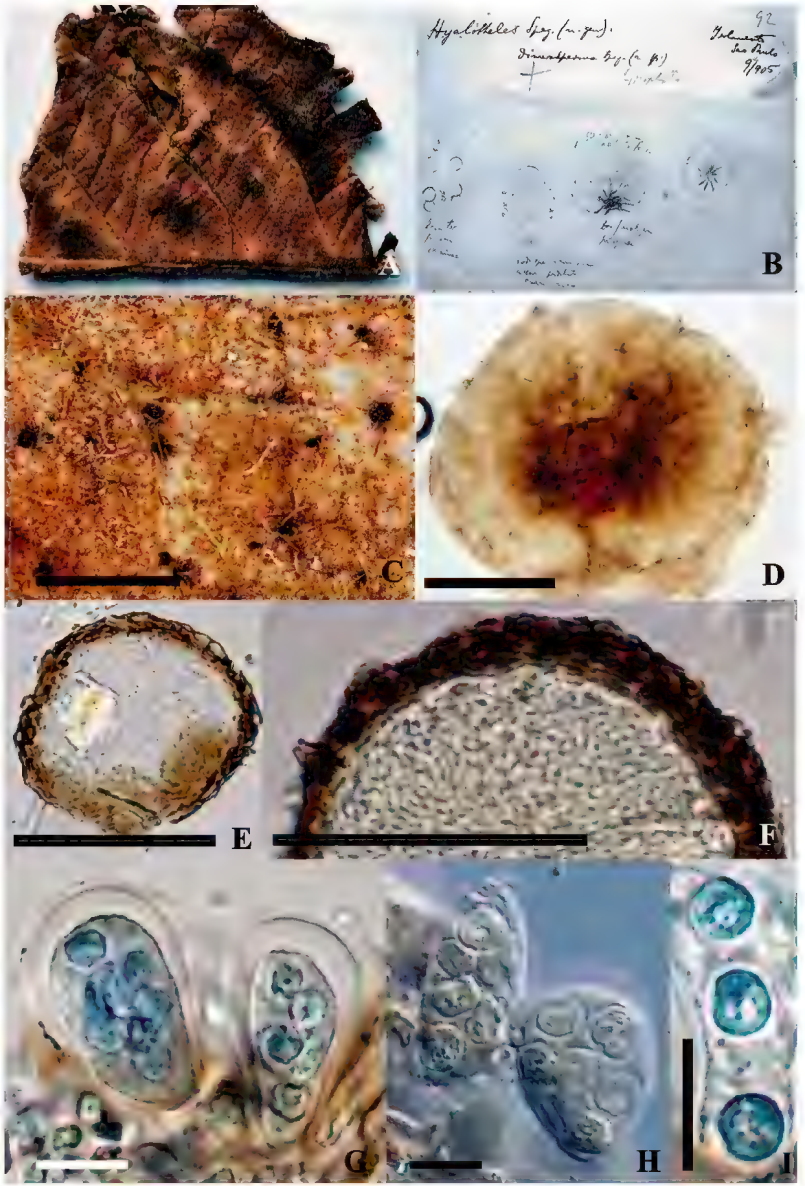


FIG. 4. *Hyalotheles dimerosperma* (holotype). A, C. Appearance of ascomata on host surface of leaf. B. Appearance of the drawing picture from the specimen. D. Ascomata in lactic acid showing the structure of dark to brown pseudoparenchymatous cells at the center and hyaline pseudoparenchymatous cells at the edge. E–F. Vertical section through ascoma in cotton blue. G–H. Asci in cotton blue. I. Ascospores in cotton blue.
Scale bars: C = 1 mm, D = 100 µm, E = 50 µm, F = 100 µm, G–H = 10 µm.

Saccardinula Speg., Anal. Soc. cient. argent. 19(6): 257 (1885).

Thalli forming raised black spots on the lower leaf surface. Individual ascomata gregarious, superficial, very easily removed from host surface, ovoid to globose, black, with the two different regions; the central region comprising dark brown radiating cells with the ascomata and the marginal parts comprising lighter brown radiating cells. Ascomata subglobose, fusing with one or more ascomata, with a single locule distributed inside each ascoma, with single asci inside each locule. Paraphyses not seen. Asci 8-spored, bitunicate, globose to oblong, without pedicel. Ascospores ellipsoid-fusiform, with a few transsepta and occasional longitudinal septa.

Anamorph: unknown.

Type species: ***Saccardinula guaranitica*** Speg., Anal. Soc. cient. argent. 19(6): 258 (1885). Fig. 5

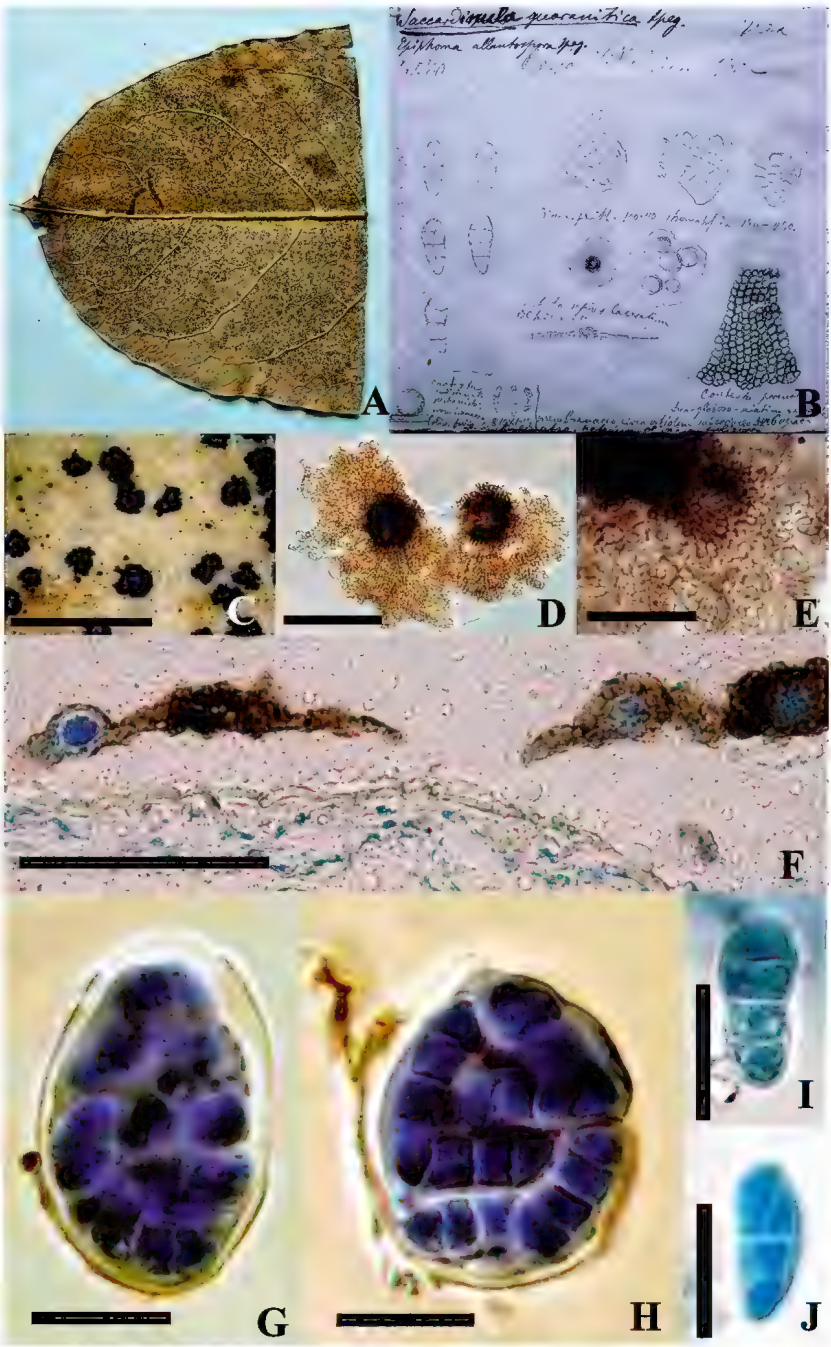
Thalli forming raised 400 µm black spots on the lower leaf surface (Fig. 5A–C). Individual thalli gregarious, superficial, very easily removed from host surface, ovoid to globose, black, with the two different regions; the central region comprising dark brown radiating cells with the ascomata and the marginal parts comprising lighter brown radiating cells to 3–7 µm in diam. (Fig. 5B,D–E). Ascomata in section 81–130 µm × 60–88 µm, subglobose, fusing with one or more ascomata, with a single locule distributed inside each ascoma, with single asci inside each locule (Fig. 5F). Paraphyses not seen. Asci 19–25 × 16–23 µm (mean = 22 × 20 µm, n = 10), 8-spored, bitunicate, globose to oblong, without pedicel (Fig. 5G–H). Ascospores 8–13 × 4–6 µm (mean = 10 × 5 µm, n = 10), irregularly arranged, ellipsoid-fusiform, 2–3 septate, constricted at the central septum, one end broadly rounded and slightly pointed at the other end, hyaline, occasionally with longitudinal septa, guttulate (Fig. 5I–J).

Anamorph: unknown.

Specimen examined: BRAZIL, Borga, Villa Rice, on leaves of *Ilex sp.*, (*Aquifoliaceae*), January1882, leg. B. Balanse (LPS 1469, holotype).

Saccardinula was erected by Spegazzini (1885) for *S. guaranitica*. Luttrell (1973) placed this genus in the "*Saccardinulaceae*" based on its ascostromata grouped in a radiate, superficial, cellular membrane. Von Arx & Müller (1975) removed this genus to *Myriangiaceae* based on its small ascomata, which are pustulate at the centre, and short (≤ 20 µm) hyaline ascospores, but Barr (1979) retained the genus in the "*Saccardinulaceae*." Lumbsch & Huhndorf (2007) placed *Saccardinula* in the family *Elsinoaceae*.

Re-examination of *S. guaranitica* shows that the ascomata greatly resemble the thyrothecia found in *Microthyriaceae* (Wu et al. 2010), although *Brefeldiella* Speg. (*Brefeldiellaceae*), which occurs on leaves and has ascomata comprising a wide area of radiating cells and a darker central raised area in which the asci form, may be more similar (Reynolds & Gilbert 2005). *Elsinoaceae* is characterized by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round or elongate ascomata composed of pale gelatinous thin-walled hyphal or pseudoparenchymatous cells and which open by unordered breakdown of the surface layers (Kirk et al. 2001). Therefore, *Saccardinula* might better be placed in either *Microthyriaceae* or *Brefeldiellaceae*, or "*Saccardinulaceae*" could be validated to accommodate it (Eriksson 1981). Ultimately only molecular sequence data will solve the problem of familial placement.

Acknowledgments

The Research Institute of Resource Insects, Chinese Academy of Forestry provided financial support to Yanmei Li PhD's study. Funds for research were provided by the Grant for Essential Scientific Research of National Non-profit Institute (no. CAFYBB2007002). The curators of the following herbaria are thanked for providing material on loan for this study: ZT, K, BISH, PAD and LPS. We also thank Professor Xiaoming Chen for financial support. K.D. Hyde acknowledges a research grant from the Biodiversity Research and Training Program (BRT R253012) and The Thailand Research Fund (BRG 5280002).

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FIG. 5 (left). *Saccardinula guaranitica* (holotype) A, C. Appearance of ascomata on the host surface of leaf. B. Drawing from herbarium specimen. D E. Ascomata in lactic acid showing the two different regions; the central region comprising dark brown radiating cells and the marginal parts comprising lighter brown radiating cells. F. Vertical section through ascoma. G H. Asci in cotton blue reagent. I–J. Ascospores in cotton blue reagent.

Scale bars: C 1mm, D 100 μm, E 50 μm, F 100 μm, G J 10 μm.

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